Louisiana Office of Public Health Laboratories	
Test Name	Shiga Toxin Positive *Escherichia coli* (STEC)
PHL Location	Office of Public Health Laboratory Baton Rouge
CPT Code	83891x9, 83894x2, 83898x36
Synonyms	EHEC, STEC, Ecoli Toxin, O157
Brief Description of Test	This test is available for when submitters get shiga toxin positive results from whole stool or GN/MAC broth and they are unable to isolate the toxin producing strain of *E. coli*. For conventional PCR from isolated culture, see *Escherichia coli* Conventional PCR from Suspect Toxin Producers. Isolation and identification of Shiga toxin producing E. coli from Toxin positive whole stool or mixed culture broth Conventional PCR for : *E. coli* mal-B promoter region *E. coli* nt 393-651 of *rfbE* (0157:H7) *E. coli* nt 454-633 of A subunit coding region of stx1 *E. coli* nt 603-857 of A subunit coding region of stx2
Possible Results	To determine *E. coli* O157 from non-O157 and to determine if *E. coli* of any kind is detected *E. coli* Detected or Not Detected O157 Detected or Not Detected To determine if genes for shiga-toxin production are present Stx1 Detected or Not Detected Stx2 Detected or Not Detected Inconclusive – No Culture Available for Confirmation
Reference Range	Not Detected
Specimen Type	Whole stool and/or Gram Negative/MacConkey broth that has tested positive for Shiga toxin by any method and *E.* coli O157 has not been isolated
Specimen Container(s):	Leak proof, screw cap slants or tubes. Do not send inoculated agar plates through the mail system.
Minimum volume accepted:	Although testing can be performed from less sample, having extra is important for referring samples to CDC if the State Laboratory is unable to isolate the specific toxin producer from the mixed flora. Approximately 1 teaspoon of whole stool or 1ml broth requested
Collection Instructions	Label specimen with Patient Name and a 2nd Unique Identifier such as a chart number or medical record number. DOB is not considered unique.

	Complete a LAB Form 93 to accompany the sample. Lab submission form must be thoroughly completed with patient's first and last name, 2nd patient identifier, gender, date of birth, date and time of collection, specimen source, test requested, submitter's name, address, fax and contact number. Additional information regarding patients' address is requested. Transport specimen to laboratory as soon as possible after collection/incubation. Keep submission forms insulated from specimens.
Storage and Transport Instructions	Whole Stool - Collect at least a BB sized sample in a leak proof screw cap container. Store and ship whole stool at 2-8°C. Send sample overnight to the Office of Public Health Laboratory Baton Rouge. Specimen must be received in the laboratory within 7 days of collection. Do not freeze. Gram Negative/MacConkey Broth - Send at least 1ml of incubated broth in a leak proof screw cap container. Ship broth at 2-8°C. Send sample to the Office of Public Health Laboratory Baton Rouge. Specimen must be received in the laboratory within 6 weeks of inoculation if stored at 2-8°C. If specimens are received greater than 6 weeks from inoculation, the lab may still attempt to isolate the suspect toxin producer. However, if a toxin producer is not isolated, the sample will be resulted as Unsatisfactory instead of Not Detected. Shiga toxin positive specimens are to be shipped refrigerated, overnight. Send sample to the Office of Public Health Laboratory Baton Rouge, 1209 Leesville Avenue, Baton Rouge, LA 70802 United Nations regulations (Division 6.2, Infectious Substances) stipulate that a verotoxigenic *E. coli* culture is a **category A (United Nations number 2814) infectious substance**, which is an infectious substance in a form capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to the substance occurs. The International Air Transportation Association (IATA) and Department of Transportation (DOT) have modified their shipping guidance to comply with this requirement. Therefore, all possible and confirmed O157 STEC and non-O157 STEC isolates and Shiga toxin--positive EIA broths should be shipped as category A infectious substances. If the identity of the infectious material being transported has not been confirmed or is unknown, but the material might meet the criteria for inclusion in category A (e.g., a broth culture that is positive for Shiga toxin or a stool culture from a patient that might be part of an O157 STEC outbreak), certain IATA regulations apply. Both IATA and DOT require that all

	persons who package, ship, or transport category A infectious substances have formal, documented training every 2 years.
Causes for Rejection	Two unique identifiers **MUST** be recorded on the specimen **AND** the Lab 93 form. A missing identifier on the tube will be an automatic rejection. If the identifiers are missing from the lab form, the submitter must be contacted and a new form with this information must be faxed back to the lab before testing/reporting will take place.
Limitations of the Procedure	PCR results should not be used as a sole test for sample identification. Negative test results may occur from improper specimen collection, handling or storage, presence of inhibitor or because the number of organisms in the specimen is below the analytical sensitivity of the test. Unless the specimen is culture, a positive test result does not necessarily indicate the presence of viable organism. Escherichia coli and Shigella spp. are difficult to differentiate with molecular methods and both may produce shiga toxin. Conventional biochemical culture confirmation is suggested. The Molecular laboratory has the capability to perform PCR for shiga toxin 1 and shiga toxin 2. We use this PCR to help identify non-O157 STEC and to isolate the non-O157 STEC from other *E. coli* normally found in stool samples. Interpretation of Final Results—Several tests for microbiology laboratories are available for the detection of STEC, and they may be used alone or in combination. No testing method is 100% sensitive or specific, and the predictive value of a positive test is affected by the patient population that a particular laboratory serves. Specificity and sensitivity might be increased by using a combination of tests. However, when test results conflict, interpretation might be difficult, especially when your result and our result are compared. Discordant results (e.g., positive immunoassay at your laboratory but negative PCR result at our laboratory) might need to be discussed among the treating physician, public health epidemiologist and both hospital and public health laboratory staff; however, the outcome of most patient illnesses (i.e., resolution of symptoms or progression to HUS) is already known by the time discordant laboratory findings are resolved.
Interfering Substances	Toxin produced by *Shigella dysenteriae* type I
References	Candrian, U., Furrer, B., Hofelein, C. Meyer, R., Jermini, M., and Luthy, J. (1991) Detection of Escherichia coli and Identification of Enterotoxigenic Strains by Primer Directed Enzymatic

	Amplification of Specific Sequences. International Journal of Food Microbiology 12, 339-352 Paton, A.W., and Paton, J.C. (1997) Detection and Characterization of Shiga Toxigenic Escherichia coli by Using Multiplex PCR Assays for stx1, stx2, eaeA, Enterohemorrhagic E. coli hlyA, rfb-O111, and rfb-O157. Journal of Clinical Microbiology 36 (2), 598-602 R.F Wang et al. 1997. A Universal Protocol for PCR detection of 13 species of foodborne pathogens in foods. Journal of Applied Microbiology 83:727-736 MMWR – Recommendations for Diagnosis of Shiga Toxin Producing *Escherichia coli* Infections by Clinical Laboratories (October 16, 2009)
Additional Information	Primers for E. coli tests were developed by AW Paton and JC Paton 1998 and Candrian et al 1991. Assay performance characteristics have been determined by the Louisiana Office of Public Health Central Lab. This test has not been cleared or approved by the U.S. Food and Drug Administration.
Release Date	3/15/2016
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